

RESEARCH ARTICLE

Inhibition of Xanthine Oxidase Activity by Ethanolic Extract of *Piperomia pellucida* L., *Acacypha indica* L. and *Momordica charantia* L.

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Abstract

BACKGROUND: Uric acid is a final result of purine catabolism, the enzymatic reactions in the body cells from amino acids or ribonucleotide dinucleotide. *Peperomia pellucida* L. (*P. pellucida*), *Acalypha indica* L. (*A. indica*) and *Momordica charantia* L. (*M. charantia*) are plants which have efficacy to reduce levels of uric acid excess. The aim of this research is to find out the effect of ethanol extract of *P. pellucida*, *A. indica* and *M. charantia* in preventing the formation of uric acid excess by inhibiting the action of the enzyme xanthine oxidase and comparing the inhibition activity of xanthine oxidase on treatments.

METHODS: The study design is experimental and conducted using the enzyme xanthine oxidase, xanthine (substrate), pH 7.5 phosphate buffer, samples (*P. pellucida*, *A. indica* and *M. charantia* ethanol extracts) and HCL as reaction breaker. Inhibition of xanthine oxidase was

determined enzymatically and unreacted xanthine was measured by UV spectrophotometer at 290 nm. The data were expressed as percent inhibition and the inhibitory concentration (IC)₅₀ were determined using linear regression of inhibition activity vs. concentration.

RESULTS: The IC₅₀ of *P. pellucida*, *A. indica* and *M. charantia* ethanol extracts in inhibiting xanthine oxidase were 19.5 ppm, 77.6 ppm and 17.8 ppm, respectively. IC₅₀ of allopurinol was 1.99 µg/ml, and negative control (combination of enzyme and substrate) has absorbance value of 0.75026.

CONCLUSION: Ethanol extract of *M. charantia* showed the most potent inhibition toward xanthine oxidase compared to the other two extracts.

KEYWORDS: xanthine oxidase, *Peperomia pellucida* L., *Acalypha indica* L., *Momordica charantia* L.

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Introduction

Uric acid is a final result of catabolism purines, the enzymatic reactions in the body cells from amino acids or ribonucleotide dinucleotide.(1,2) The occurrence of hyperuricaemia, or excessive increase of uric acid in the

blood, will cause gout disease.(3,4) Xanthine catalyzed by the enzyme xanthine oxidase will form uric acid. Xanthine oxidase, which plays an important role in the purines catabolism, is found in liver and muscle cells, but can not be found in the blood.(5) The normal value of uric acid level is 3.5 to 7.0 mg/dL for man and 2.5 to 6.0 mg/dL for women.

The intake of foods containing high purines will increase uric acid levels. The use of *Peperomia pellucida* L. (*P. pellucida*), *Acalypha indica* L. (*A. indica*) and *Momordica charantia* L. (*M. charantia*) in lowering uric acid levels is very beneficial. Purine and pyrimidine nucleotides synthesized *in vivo* at a pace that is consistent with physiological needs. The purine bases are then oxidized to uric acid, which may be absorbed and excreted in the urine. While little or no dietary purine or pyrimidine is incorporated into tissue nucleic acids. There are three processes that have roles in the biosynthesis of purine nucleotides. These three processes, sorted from the most important, are the synthesis of intermediates amphibolic (synthesis *de novo*), phosphorylation of purine and phosphorylation of purine nucleoside.

Adenosine first undergo deamination into inosine by the help of adenosine deaminase enzyme.(6) Hypoxanthine and xanthine guanine subsequently formed in a reaction catalyzed by enzyme xanthine oxidase and guanase, respectively. The oxidized xanthine is turned into uric acid in the second reaction catalyzed by the enzyme xanthine oxidase. Thus, xanthine oxidase is an essential place for pharmacological intervention in patients with hyperuricemia and gout.(7,8)

These days, many number of plants have been proved empirically or by scientific testing and used as alternative treatments.(9) Some plants have been proved to be effective in lowering the level of uric acid excess, those are *P. pellucida*, *A. indica* and *M. charantia*.

Other efficacy of *P. pellucida* is its ability to cope with headache, abdominal pain, abscesses, ulcers, kidney disorders, acne, dermatitis, minor burns and pain in rheumatoid arthritis.(10,11) Extract of *P. pellucida* 1g/kg bw/day in rats induced potassium oxonate three hours before treatment, suggesting that *P. pellucida* can be used as an alternative medicine for uric acid.(12)

Beside it effects as an anti-uric acid, *A. indica* also has efficacy as an anti-inflammatory, diuretic, laxative and hemostasis. Extract of *A. indica* 2.7g/200g bw/day can effectively lower uric acid level in rats, which induced potassium oxonate one hour before treatment.(13)

Another plant that is often used in the treatment of uric acid is *M. charantia*.(14) Therefore, this study was done to find out the ethanol effects of extract *P. pellucida*, *A. indica* and *M. charantia* in inhibition of the formation uric acid by using the *in vitro* treatment method.

Methods

The design of this study was experimental study with post-test group design, using the three treatment groups with each repetition three times. For the manufacture of blanks solution, 2.8 mL of potassium phosphate buffer pH 7.5 was inserted then 0.1 mL of xanthine was added. For control sample (allopurinol), 2.9 mL potassium phosphate buffer at pH 7.5 and 0.1 mL sample were inserted. Meanwhile for the negative control, 2.7 mL of potassium phosphate buffer pH 7.5, 0.1 mL xanthine and 0.1 mL xanthine oxidase were inserted. As for the samples, 2.6 mL of potassium phosphate buffer at pH 7.5, 0.1 mL xanthine, 0.1 mL xanthine oxidase and 0.1 mL of sample were inserted. After all solution inserted in to the cuvette, it was incubated for 10 minutes. After the incubation, 0.1 mL of HCl was added as the breaker of enzyme reaction. The absorbance measurement is performed using a spectrophotometer at a wavelength of 290 nm.

Materials used were botanicals *P. pellucida*, *A. indica* and *M. charantia* which were obtained in Makassar, distilled water, 96% ethanol, DMSO, $K_2HPO_4 \cdot 3H_2O$, $KH_2PO_4 \cdot 3H_2O$, NaOH, HCl, allopurinol, xanthine and xanthine oxidase (Sigma, St. Louis, USA). The preparation of *P. pellucida*, *A. indica* and *M. charantia* was performed at the Biology Laboratory of Halu Oleo University. Those plants were harvested, sorted and then cut into pieces. Lastly they were dried and then stored in airtight plastic bags until being used.

Preparation of Extract

Initially, the herbs were cleaned, washed with water, and cut into small pieces. After it was dried, then it was placed in an open space with good circulation and no exposure to direct sunlight. It could not be placed in high temperature because the exposure of direct sunlight causes damage to the active component in herbs.

The dried herbs then extracted by maceration method, which was soaking the dried herbs in a solvent of ethanol 96% for 24 hours, then filtered it with filter cloth and soaked it in ethanol 96% until it was extracted perfectly. After that, the solvent is evaporated on a rotary evaporator and the remaining ethanol aerated to obtain a thick extract with a concentration of 100%.

Preparation of Potassium Phosphate Buffer pH 7.5

Potassium phosphate buffer at pH 7.5 was manufactured at 25°C, by mixing 0.68045 g of $\text{KH}_2\text{PO}_4 \cdot 3\text{H}_2\text{O}$ and 4.352 g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, which was dissolved in 500 mL of deionized aqua.

Making Xanthine 0:15 mM

The substrate was created by dissolving 22.5 mg of xanthine using 10 mL phosphate buffer from previous solution. Then the substrate was sufficed by taking 1 mL to 100 mL.

Xanthine Oxidase Inhibition Test *in Vitro*

Power test inhibition of ethanol extract *P. pellucida*, *A. indica* and *M. charantia* was performed at the optimum condition which refers to the Sigma product sheet. That optimum condition refers to a 10 minutes incubation time at the temperature of 25°C, pH 7.5, a wavelength 290 nm, xanthine oxidase concentration of 0.1 unit/mL and xanthine concentration of 0.15 mM.

Total of 2.6 mL phosphate buffer was put in a test tube, followed by 0.1 mL extract. Then 0.1 mL of 0.15 mM xanthine and 0.1 mL of 0.1 unit/mL xanthine oxidase added to the mixture, and incubated at a temperature of 25°C for 10 minutes.

After the incubation, the mixture was immediately added by 0.1 mL of 1 M HCl to stop the reaction. The mixture was measured using UV absorption at a wavelength of 290 nm to see how big the rest of xanthine unreacted in the test sample. The obtained power inhibition test result was compared to allopurinol result.

Results

Table 1 shows the result of xanthine oxidase inhibitory activity of the ethanol extract of *P. pellucida*, *A. indica*, *M. charantia* and allopurinol (as positive control). It shows the concentration, log concentration, inhibition percentage, probit and inhibitory concentration (IC_{50}) value from each sample extract and the positive control. IC_{50} values from each extract are obtained to determine the effect and comparison of enzyme xanthine oxidase inhibitory activity. IC_{50} values were 19.5 $\mu\text{g}/\text{mL}$, 77.6 $\mu\text{g}/\text{mL}$, 17.8 $\mu\text{g}/\text{mL}$ and 1.9 $\mu\text{g}/\text{mL}$ for *P. pellucida*, *A. indica*, *M. charantia* and allopurinol, respectively.

Discussion

Plants used in this research are *P. pellucida*, *A. indica* and *M. charantia* which were extracted by maceration method. Maceration process was done by soaking the sample in a container using liquid extraction of 96% ethanol for 3x24 hours with occasional stirring, then being filtered. During the soaking, extraction liquid would penetrate the cell wall and enter the cell. Maceration method was chosen to avoid damage because the chemical constituents are not resistant to heating and provide good result and selectively extracted.

The primary data of xanthine oxidase inhibition assay results was obtained from the treatment group and a control group in Hasanuddin University Pharmaceutical Chemistry Laboratory. The test sample consisted of *P. pellucida*, *A. indica* and *M. charantia* ethanol extract, as well as the positive control which was allopurinol and negative controls which was combination of enzyme and substrate. Data were analyzed to determine the percentage of inhibition values, followed by linear regression statistical test and the determination of the inhibitory activity of enzyme xanthine oxidase samples to determine the IC_{50} value.

Purine bases converted into hypoxanthine by xanthine oxidase are converted into xanthine and uric acid. Uric acid is formed and then discharged through urine, gastrointestinal tract and partially backfilled in tissue in the form of monosodium uric (MSU) crystals, which over time will accumulate and form microtophy. While the inflammatory process that occurs due to the deposition of uric acid crystals in the tissues around the joints, gout is also a term used for a group of metabolic disorders characterized by an increased concentration of uric acid, or also called hyperuricemia.

Enzymes can not work without the substrate, so does enzyme xanthine oxidase that will only work on an appropriate substrate, which is xanthine.

Xanthine oxidase catalyzes xanthine by addition of distilled water and oxygen into uric acid and hydrogen peroxide. Hydrogen peroxide has the ability to diffuse into and penetrate the cell membrane that can cause damage to cells located far from where hydrogen peroxide was formed.

Table 1. Determination of inhibition percentage and IC₅₀*

Sample	Concentration (ppm)	Log Concentration	Inhibition Percentage (%)	Probit	IC ₅₀ (µg/ml)
<i>P. pellucida</i>	200	2.3	21.01	4.19	19.5
	100	2.0	35.53	4.62	
	50	1.7	43.86	4.84	
	25	1.4	48.61	4.96	
	12.5	1.1	50.44	5.01	
<i>A. indica</i>	200	2.3	60.75	5.27	77.6
	100	2.0	50.52	5.01	
	50	1.7	46.76	4.91	
	25	1.4	40.86	4.77	
	12.5	1.1	29.31	4.46	
	6.25	0.8	7.03	3.52	
<i>M. charantia</i>	100	2.0	23.99	4.29	17.8
	50	1.7	42.44	4.81	
	25	1.4	47.32	4.93	
	12.5	1.1	52.58	5.07	
	6.25	0.8	63.56	5.35	
Allopurinol	5	0.7	39.55	4.74	1.99
	2.5	0.4	45.04	4.87	
	1.25	0.1	51.75	5.04	
	0.625	-0.2	67.98	5.47	
	0.3125	-0.5	77.28	5.75	
	0.15625	-0.8	84.72	6.03	

Measurement of xanthine oxidase inhibitory activity was performed according to the Bergmeyer "Method in Enzymology". The samples were measured using spectrophotometer based on the decrease in absorption at 290 nm. Measurement of the activity of xanthine oxidase was done at pH 7.5, because if the atmosphere is too acidic or alkaline, it can cause loss of xanthine oxidase activity.

In vitro testing was conducted to observe the activity of the enzyme xanthine oxidase on the formation of urate crystals. A decrease in the activity of xanthine oxidase in the ethanol extract of *P. pellucida*, *A. indica* and *M. charantia* will reduce or prevent the formation of urate crystal and hydrogen peroxide. This test was done to determine the extent of the effects that have been caused by the extracts.

Testing IC_{50} previously used to extract samples contained some activity, *P. pellucida* extracts showed antioxidant activity (15), inhibitory effects on three cancer cell lines and has a weak suppressor activity in HL-60 cells (16). The methanol extract of *A. indica*, showed anticancer activity against cell-NCIH187 small lung cancer, showed significant anticancer activity against HT-29 colon cancer cells (17), and the test protein denaturation, 37 $\mu\text{g/mL}$ in action proteinase inhibitory and 18 $\mu\text{g/mL}$ for anti-hyaluronidase activity (18). As well as the methanol extract of *M. charantia*, dose-dependent inhibit the activity of sucrase most powerful of the intestinal mucosa (19), and has disaccharidase-inhibitory activity on the improvement of blood glucose and serum (20).

Each extract was tested in the same concentration, which was 60 mg. After determining the concentration, measurement of absorbance obtained from each concentration is the value to be incorporated into the linear regression equation to obtain IC_{50} value.

The information of IC_{50} value in this research is aimed to determine the effect and comparison enzyme xanthine oxidase inhibitory activity of the test sample. It was obtained ethanol extract of *P. pellucida* $IC_{50} = 19.5 \mu\text{g/mL}$, the ethanol extract of *A. indica* $IC_{50} = 77.6 \mu\text{g/mL}$, the ethanol extract of *M. charantia* $IC_{50} = 17.8 \mu\text{g/mL}$, allopurinol (positive control) $IC_{50} = 1.99 \mu\text{g/mL}$ and the negative controls (combination of enzyme and substrate) has absorbance value of 0,75026. IC_{50} value was inversely related to the inhibition of xanthine oxidase activity, the smaller the IC_{50} value, the greater the inhibition activity of xanthine oxidase (Table 1).

Allopurinol was used as a positive control to determine differences of concentration and absorption produced by extracts and allopurinol. From the test sample, it is known that extract of *M. charantia* provides inhibitory effect on the formation of MSU crystals.

Conclusion

From this study, it can be concluded that ethanol extract of the *P. pellucida*, *A. indica* and *M. charantia* influence the inhibition of MSU crystals formation. It was found that there is a xanthine oxidase inhibitory activity of the samples tested. The smaller the IC_{50} value means the greater the inhibition of xanthine oxidase activity of that samples. IC_{50} values for extracts of *M. charantia*, *P. pellucida* and *A. indica* are 17.8 mg/mL, 19.5 mg/mL and 77.6 mg/mL, respectively.

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